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Myeloid Hyperplasia in the SENCAR Mouse: Differentiation from Granulocytic Leukemia

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The term myeloid hyperplasia has been used interchangeably with many other terms to describe an increased production of granulocytes, megakaryocytes, and erythrocytes in the spleen and other organs in the mouse. This process is occasionally misdiagnosed as granulocytic leukemia. This paper reviews some of the terms used interchangeably with myeloid hyperplasia and describes criteria that can be used to differentiate myeloid hyperplasia from granulocytic leukemia. Additionally, the results of a study in which myeloid hyperplasia was induced following the formation of skin tumors in SENCAR mice is discussed. In this study, positive correlations were found between skin lesions, the spleen weight, and histologic appearance of the spleen. The liver rarely showed microscopic changes of myeloid hyperplasia unless the spleen weighed at least 1.0% of the body weight.

Introduction

Many strains of mice, including the SENCAR stock, respond to inflammatory stimuli, especially of the skin, by greatly expanding the granulocyte pool with an increased production of granulocytes in various organs of the hematopoietic and lymphoreticular systems. Frequently, proliferation of granulocytic cells is accompanied by erythropoiesis, megakaryocytosis, plasmacytosis, and reticuloendothelial cell hyperplasia in various organs of these systems.

Many diagnostic terms, such as myeloid hyperplasia, leukemoid response, myeloid metaplasia, extramedullary hematopoiesis (EMH), and granulopoiesis have been used interchangeably to describe this phenomenon. However, in their pure form all these terms are not synonymous. For instance the term leukemoid response refers to an extreme elevation of the white blood cell count in the peripherial blood (*1–3*). Myeloid metaplasia and EMH refer to the formation and development of blood cells outside the bone marrow (*1,3*), and granulopoiesis is the formation of granulocytes (*1,3*). Myeloid hyperplasia, as we use the term, indicates a relative (mild to severe) increase in the formation and development of granulocytes above that level which is considered normal and is usually associated with an inflammatory tissue reaction elsewhere. The use of these terms interchangeably often results in confusion among those trying to describe and record similar lesions.

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The diagnosis of myeloid hyperplasia is confounded by the fact that microscopically observed lesions may mimic those of granulocytic leukemia (*1,4–7*). Although relatively rare when compared to neoplasms of lympoid cells, granulocytic leukemia does occur and must be differentiated from severe hyperplasia of granulocytic cells (*4,8*). The importance of separating myeloid hyperplasia from granulocytic leukemia can not be overemphasized. Many times, the animals in which the hyperplastic or neoplastic cells are seen have been given a variety of test materials of potentially significant environmental impact. Misdiagnosis under these circumstances could have a profound effect on the outcome of the study and the compounds involved.

In an extensive review of the hematopoietic and lymphoreticular systems, Dunn details the criteria used to differentiate granulocytic leukemia and myeloid hyperplasia, referred to as "nonmalignant extramedullary myelopoiesis" in her article. These criteria, as well as those of other authors are outlined in Table 1 (*1,4,5,7*). The most important criterion appears to be the presence or absence of various stages of granulocyte maturation (*1,4,5,7*). However, in the mouse it is difficult to identify all phases of development and usually only three stages can be distinguished. The young or early forms are larger cells with basophilic cytoplasm, with or without azurophilic granules and a round or early ring-form nucleus with finely stippled chromatin. The old or mature form has a pale basophilic or neutrophilic cytoplasm with a ring form nucleus showing early indentation or fully developed segmentation and coarsely distributed chromatin. The intermediate forms include all the remaining cell stages (*1,5*).

In this report we will attempt to clarify further some of the terminology, report the results obtained from

Table 1. Myeloid hyperplasia vs. granulocytic leukemia in the mouse.

Myeloid hyperplasia	Granulocytic leukemia
All stages of granulocyte development present	Usually immature cells with one stage predominating
Erythropoietic activity usually present	Erythropoietic activity absent
Megakaryocytes are usually numerous	Megakaryocytes are few and present in organs where they are normally found
Frequently associated with inflammation	Not usually accompanied by inflammation
Cells are not invasive	Cells are invasive
Normal tissue of the spleen is relatively unaffected	Normal tissue of the spleen is extensively replaced
Blood is normal or leukocytosis with mature forms	Blood usually contains immature forms and may have high white blood count
Hemorrhages are absent	Hemorrhages are frequent
Not transmissible	Transmissible
Not transplantable	Transplantable
Can occur at any age	Usually occurs in mice over 12 months old
When the lymph nodes are involved the cells are usually confined to the medulla	When the lymph node is involved the cells are not confined to the medulla
Bone marrow is involved late	Bone marrow is involved early
Bone marrow involvement does not result in destruction of the bone	Bone marrow involvement results in destruction of the bone

examination of cases on file, and provide some data from a study designed to induce myeloid hyperplasia.

Materials and Methods

Reports from three studies at the Health Effects Research Laboratory/Toxicology and Microbiology Division (HERL/TMD) at the Environmental Protection Agency (EPA) were examined. Slides were evaluated from eight animals that had been diagnosed as having myeloid (granulocytic) leukemia, leukemoid reaction, extramedullary hematopoiesis, and/or granulopoiesis in one or more of the following organs: liver, spleen, and lymph node.

In addition, a study, using 160 SENCAR mice divided into four groups of 40 animals each, was performed to induce myeloid hyperplasia. Groups 1, 2, and 3 were initiated with one topical application of benzo(a)pyrene (2.0 mg/kg). Promotion was started 5 days later with topical application of 12-O-tetradecanoylphorbol-13-acetate (TPA) 3 times a week for 15 weeks (7 weeks at 2.0 μg TPA in 0.2 mL of acetone per mouse followed by 8 weeks of 4.0 μg TPA in 0.2 mL of acetone per mouse). Group 4 was treated topically with 0.2 mL of acetone per mouse at the time of initiation, following the same schedule as that used for the promotion of groups 1 through 3. During week 19, the mice in group 2 were anesthetized with ether, and up to two tumors per animal were removed. The incisions were closed and the mice were allowed to recover. During the same week, the mice from group 1 and half of the mice (20 animals) from group 4 (labeled group 4ES) were sacrificed in a CO_2 chamber. The weights of the body, liver, and spleen were recorded, and selected tissues were placed into 10% buffered neutral formalin (BNF) for subsequent microscopic examination. During week 29 the remaining mice in groups 2, 3, and 4 (now labeled group 4LS) were sacrificed, the weights of the body, liver, and spleen were recorded, and selected tissues were placed in 10% BNF for subsequent microscopic examination. Tissues were processed, embedded in paraffin, sectioned at 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination.

Results

Slides from a previous HERL/TMD study were evaluated using the criteria listed in Table 1. Sections from spleens that had been diagnosed as having granulocytic leukemia, leukemoid response, or EMH were examined. There was no outstanding feature present to distinguish granulocytic leukemia from the leukemoid response. In fact, the granulocytic cell population of the spleen diagnosed as leukemoid response appeared to have the most immature cell population with the most uniform stage of development, characteristics usually associated with granulocytic leukemia. The spleens diagnosed as having EMH in general could be readily differentiated from those with granulocytic leukemia or leukemoid response. They usually had more erythropoietic foci and fewer numbers of granulocytes. However, one mouse with EMH was histologically similar to one diagnosed with leukemoid reaction, except that the former had a greater number of mature granulocytes. Based on previously stated criteria and definitions, it appears that these cases should have been diagnosed as EMH or myeloid hyperplasia.

Liver sections diagnosed as having granulocytic leukemia, leukemoid response, or granulopoiesis were also evaluated. Again there were no discernable differences between the livers of mice with leukemoid response and those with granulocytic leukemia. There was extensive involvement of the liver primarily at the tips of the lobes in all these mice. In two animals, one with granulocytic leukemia and one with leukemoid response, necrosis of hepatocytes was accompanied by intense infiltration of granulocytes. However, in all instances, all stages of granulocyte development were present, as were a few megakaryocytes scattered in sinusoids. Occasionally, small erythropoietic foci were present in livers of mice with leukemoid response. A large number of mature and a few immature granulocytes were found within the lumens of blood vessels in the liver of one mouse diagnosed as having granulocytic leukemia. When the liver is evaluated alone, the changes observed may lead to a diagnosis of granulocytic leukemia. However, when these changes are considered with changes seen in other tissues, a diagnosis of granulocytic leukemia may not be appropriate.

The livers of mice diagnosed with granulopoiesis were less severely affected and contained scattered foci of granulopoiesis in sinusoids and adjacent to blood ves-

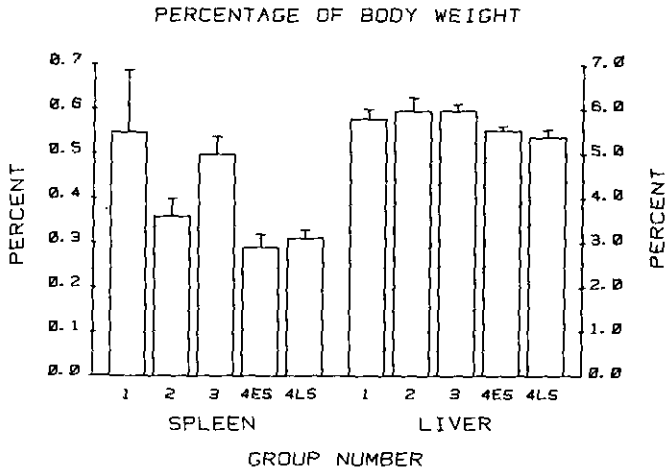

FIGURE 1. Spleen and liver weights by group. Spleen and liver weights expressed as a percentage of body weight compared by groups.

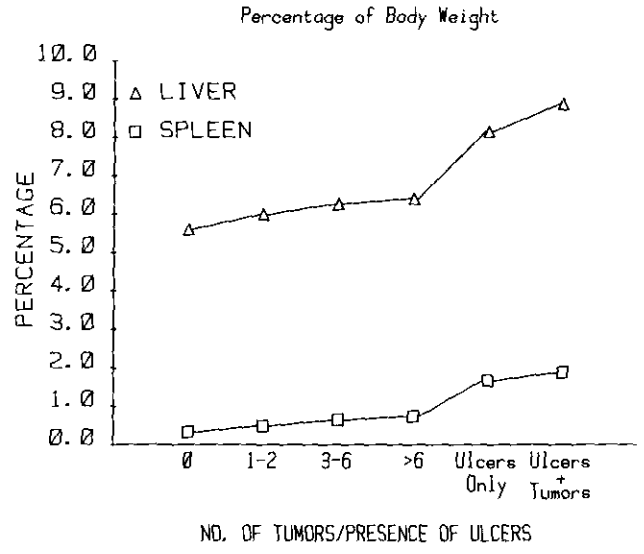

FIGURE 2. Spleen and liver weights by skin lesions. Spleen and liver weights expressed as a percentage of body weight compared by the number of skin tumors present and the presence of dermal ulceration not associated with skin tumors.

sels. Occasionally small foci of erythropoiesis were present, but megakaryocytes were not consistently found. The granulocytes present in the livers were a mixture of all stages of granulocyte development. The major difference between these livers and those described previously was in the number of cells and the amount of hepatic tissue involved.

The data from our study designed to induce myeloid hyperplasia showed a difference among the groups when the mean spleen weights per group, expressed as a percentage of the body weight, were compared (Fig. 1). The mean spleen weight of group 1, the treated group sacrificed early, and group 3, the treated group sacrificed at the end of the study, were essentially the same. Mean spleen weights in group 4ES, the half of the vehicle control group sacrificed early, and in group 4LS, the half of the vehicle control group sacrificed at the end of the experiment, were essentially the same, and both were much lower than groups 1 and 3. The mean spleen weight of group 2, the treated group with the tumors surgically removed and sacrificed at the end of the study, was lower than that in groups 1 and 3 but slightly higher than those in groups 4ES and 4LS. When the same comparisons were made using the liver weight expressed as a percentage of the body weight, no difference was found among any of the groups (Fig. 1).

When the mean liver and spleen weights, expressed as a percentage of the body weight, were evaluated based on the number of skin tumors and the presence of dermal ulcerations not associated with skin tumors, a definite pattern was seen (Fig. 2). The number of skin tumors was also used as a measure of inflammation. In general, as the number of tumors increased so did the amount of associated inflammation (ulcerative, but not always suppurative). There was an increase in the mean weight of the liver and spleen as the number of tumors increased. The liver and spleen weights were highest when dermal ulcers, not associated with skin tumors, were present. The increases in liver and spleen weights parallel each other in mice that had only skin tumors. In mice that had dermal ulcerations not associated with tumors, the difference between the liver and spleen weights was much greater. This difference was attributable to the greater increase in liver weight.

Spleen weights appeared to increase as a result of increased number of cells of the erythrocytic and granulocytic series in the spleen. Usually this increase was not detected microscopically until the spleen weighed 0.5 to 0.7% of the body weight. In contrast to the spleen, the mild increases in the weight of the liver were not as readily attributed to cells of the granulocytic or erythrocytic series and in many cases, no histologic evidence for these increases were found. No granulocytic precursors were seen in the liver unless the spleen was also involved, except in two instances. In most cases the spleen was severely affected and accounted for greater than 1% of the body weight before the liver was involved. Pathological changes in the lymph nodes were not observed unless the spleen was severely infiltrated by granulocytic cells.

The spleen and lymph nodes were consistently grossly enlarged in animals that had a large number of tumors or had dermal ulcerations not associated with skin tumors (Figs. 3 and 4). Microscopically, spleens from mice with no skin lesions had very few randomly scattered granulopoietic foci in the red pulp. These foci contained relatively few granulopoietic cells compared to foci from spleens of animals bearing skin lesions. In contrast to the random distributions in the normal spleen, granulopoietic foci first appeared just beneath the splenic capsule and adjacent to the trabeculae in the red pulp in animals with skin lesions (Fig. 5). As the number of granulocytic cells increased, these foci expanded and formed a rim of proliferating cells beneath the capsule

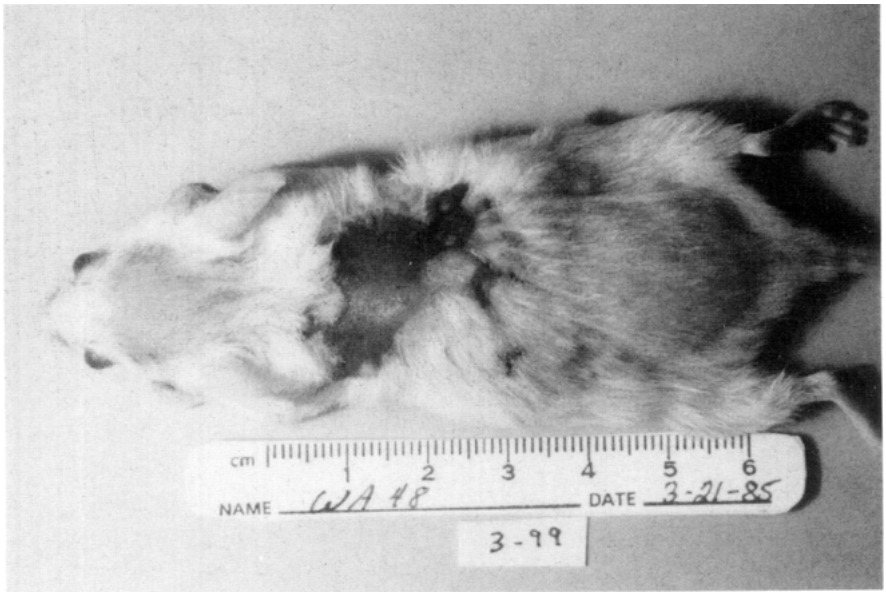

FIGURE 3. Dermal ulceration. Note the large area of dermal ulceration over the anterior one-third of the back following treatment with 2μg TPA three times per week for 15 weeks and 4 μg TPA three times per week for 8 weeks.

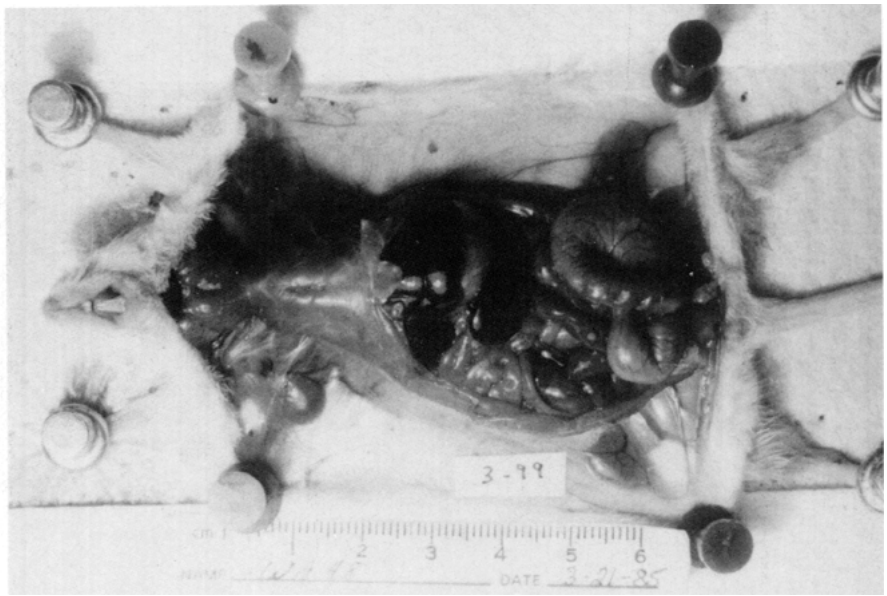

FIGURE 4. Enlarged spleen and peripheral lymph nodes. Mouse had extensive skin tumors.

and around the trabeculae (Fig. 6). In severe cases, granulocytes and their precursors began filling the red pulp (Fig. 7). In all instances, megakaryocytes and foci of erythropoiesis were scattered among the proliferating granulocytes throughout the red pulp. Although the white pulp changes were not critically evaluated in this study, no extreme differences were noted among the groups. To formulate accurate conclusions on changes occurring in the white pulp would require a complete reevaluation of the spleens. In the liver, foci of granulopoiesis with occasional megakaryocytes were found adjacent to hepatic and portal vessels as well as in sinusoids (Fig. 8). The reaction in the lymph node was characterized by extensive plasma cell infiltration of the medulla with or without granulocytes and their precursors in medullary cords and sinuses (Fig. 9). Reticuloendothelial cell hyperplasia was an inconsistent finding. These changes occurred with approximately equal frequency and severity in the inguinal and axillary lymph nodes. The mesenteric lymph nodes were infre-

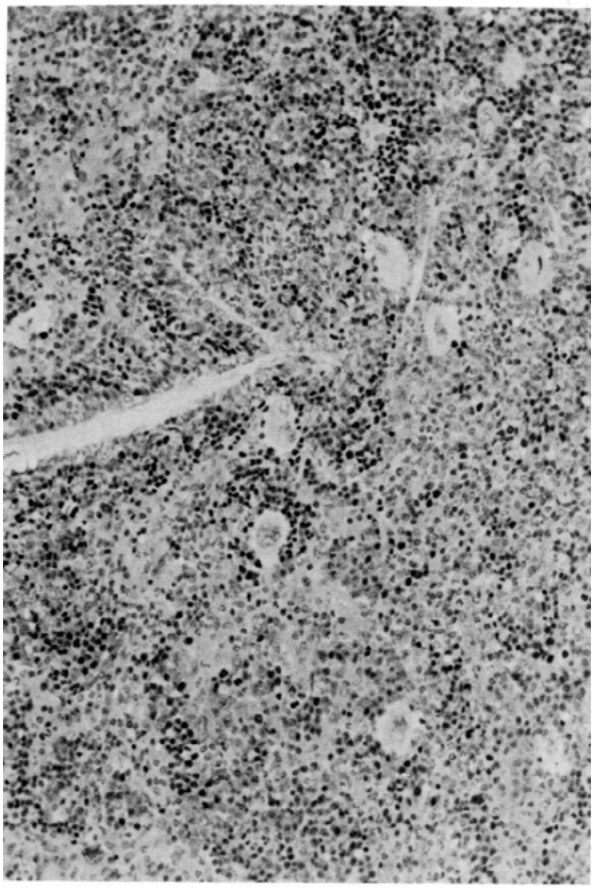

FIGURE 5. Mild myeloid hyperplasia in the spleen. Note granulopoietic foci adjacent to trabeculae, megakaryocytes, and foci of erythropoiesis.

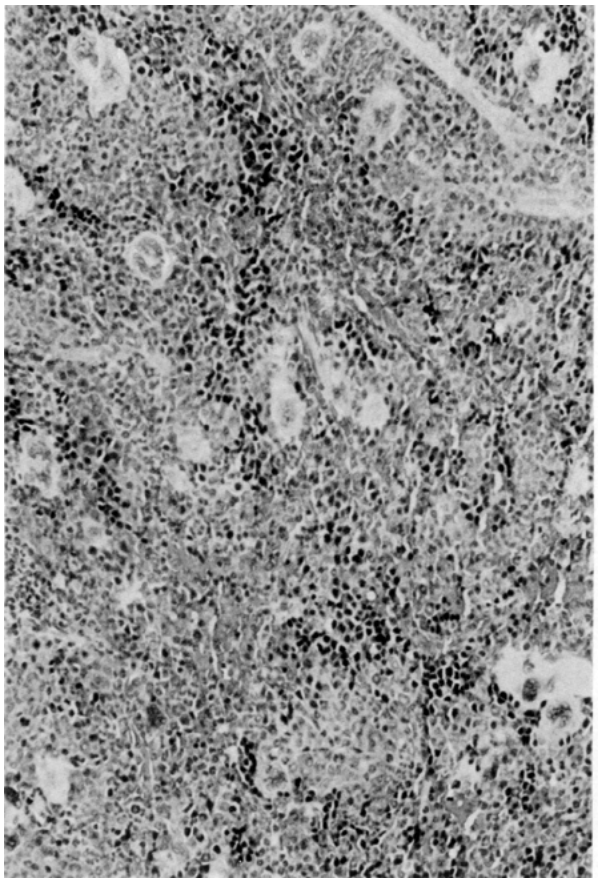

FIGURE 6. Moderate myeloid hyperplasia in the spleen. Note the rim of proliferating granulocytic cells adjacent to trabeculae, megakaryocytes, and foci of erythropoiesis.

quently involved. Only rarely were changes seen in the mesenteric lymph node without involvement of the axillary and/or inguinal lymph node.

Discussion

Granulocytic leukemia and myeloid hyperplasia are not always easily distinguished from one another, especially when only one or two tissues are available for microscopic examination. In granulocytic leukemia the cells are immature, usually with one stage predominating. However, in myeloid hyperplasia, all stages of development can be found (*1,4,5,7*). Other commonly used criteria for differentiating these two conditions are the presence of erythropoiesis, megakaryocytes, and an inflammatory reaction. These conditions are typically found with myeloid hyperplasia but are not usually associated with granulocytic leukemia (*1,5,7*). When the number of available tissues is limited, the presence of capsular invasion, the age of the mouse, and the presence of hemorrhages should also be considered when making a diagnosis (*1,5,7,8*).

However, a thorough macroscopic and microscopic examination of a complete set of tissues is more likely to provide information that will enable one to separate these two conditions (*5*). Items that should be noted during the necropsy include: the presence of inflammatory conditions; the size of the liver, spleen, and lymph nodes; the weights of the liver and spleen; and presence or absence of hemorrhage. Tissues to be collected to facilitate a diagnosis include the spleen, liver, peripherial lymph nodes, bone marrow (formalin-fixed and fresh smears), kidneys, adrenals, salivary glands, and ovaries. If the live animal is available, additional techniques such as collection of blood for a complete leukocyte count with a differential count and the harvesting of tissue for transmission and transplantation studies should be done (*1,5,7*).

The extent of organ involvement can only be determined if a full set of tissues is available. This information can be most useful in differentiating these two conditions because the order in which the organs are affected is different in each. In myeloid hyperplasia, the bone marrow is one of the first tissues to be affected. This order of occurrence is in contrast to granulocytic leukemia in which bone marrow invovlement occurs late

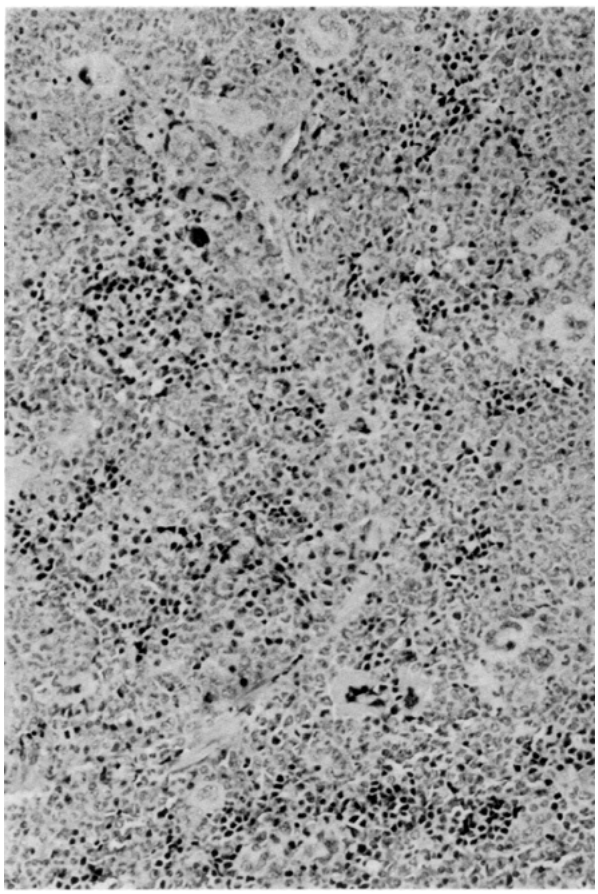

FIGURE 7. Severe myeloid hyperplasia in the spleen. Note diffuse granulopoiesis involving the red pulp, megakaryocytes, and foci of erythropoiesis. The white pulp was considered to be within normal limits.

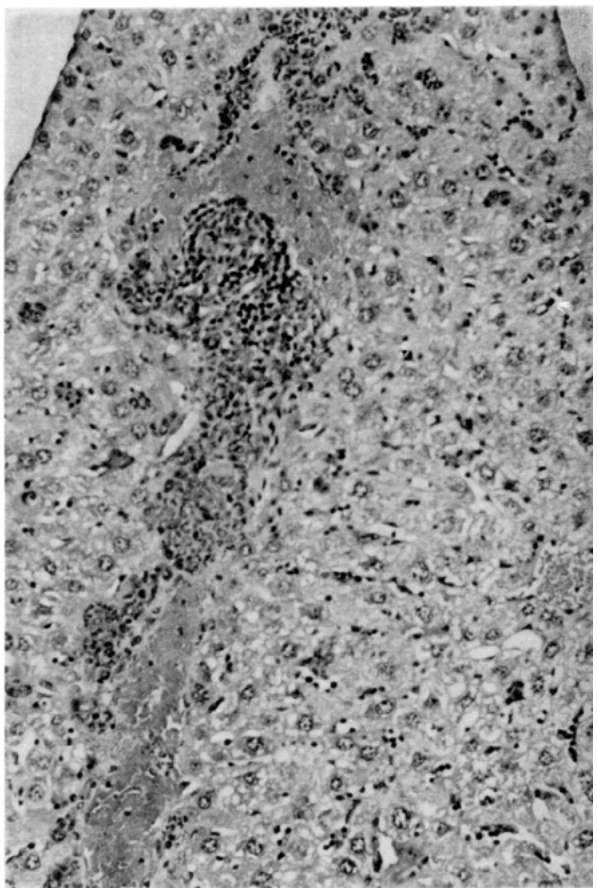

FIGURE 8. Myeloid hyperplasia in the liver. Note foci of granulopoiesis adjacent to vessels as well as in sinusoids.

in the course of the disease and may be associated with bone destruction. Additionally, in granulocytic leukemia, the spleen is the first organ affected followed by the liver. Neoplastic cells infiltrate the lymph nodes, bone marrow, and other organs during the later stages of the disease (*5,7,8*).

The study we conducted was designed so that no tumors or dermal ulcerations would be present on any animal in group 2 at the end of the experiment. We discovered the amount of tissue that had to be excised to ensure complete removal of the skin tumors was so great that no more than two tumors could be removed from any one mouse. Therefore, when evaluating of the group data for groups 1, 2, and 3, only those animals with one or two skin neoplasms were used. In all, 29 mice in group 1, 32 mice in group 2 and 28 mice in group 3 either died early or did not meet the criteria required for evaluation as outlined above and were thus eliminated from the evaluation.

The presence of myeloid hyperplasia at week 19 and week 29 was reflected by the elevated mean splenic weight of group 1 and group 3, respectively. In both groups, the increased mean splenic weight correlated microscopically with an increase in the number of cells of the granulocytic and erythrocytic series. The absence of an increased mean splenic weight in the tumor-free, vehicle control mice (group 4) at the early and the terminal sacrifice suggests that the presence of skin tumors provided the stimulus for the induction of the myeloid hyperplasia. The low mean splenic weight of group 2 at the terminal sacrifice suggests that myeloid hyperplasia was reversed by the removal of the tumors. The lack of correlation between increased mean liver weight and histologic evidence of myeloid hyperplasia in the liver in mice having one or two skin tumors may have been due in part to the low level of liver involvement or the lack of uniform changes within the lobes of the liver.

As the number of skin tumors increased, there was a trend toward greater liver and spleen weights. The marked rise in liver and spleen weights when dermal ulcerations not associated with skin tumors were present suggests the associated inflammatory reaction was a positive stimulus for the initiation of myeloid hyperplasia. Increased production of granulocytes as a result of an inflammatory lesion is well documented in the literature (*2,7,9,10*). Increased spleen weights in mice with dermal ulcerations correlated microscopically with the presence of myeloid hyperplasia. This finding was to be expected based on reports in the literature (*7*).

FIGURE 9. Myeloid metaplasia and plasmacytosis in a lymph node. Note the severe infiltration of the lymph node by plasma cells. Scattered foci of granulopoiesis and megakaryocytes are also present.

However, the greater increase in the liver weight when compared to the spleen weight in the presence of dermal ulcerations was not expected and is not easily explained.

In conclusion, myeloid hyperplasia usually results from an increased demand for granulocytes and may affect the spleen, liver, and lymph nodes, as well as other organs. It is most commonly induced by inflammatory conditions. Histologically, the spleen, liver, lymph nodes, and occasionally other organs may be infiltrated by proliferating granulocytes that can sometimes be mistaken for neoplastic cells associated with granulocytic leukemia. A number of microscopic criteria can be applied to help distinguish between the two conditions. However, they are much easier to differentiate if a complete diagnostic workup is performed.

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